General Biochemistry

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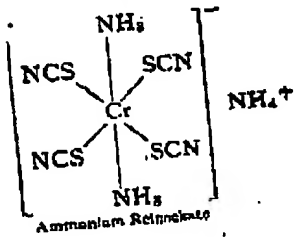
tructural Units of Proteins

of chemical substances some- since they were first studied rner during the period 1900– ringly soluble salt with proline ilate may therefore be obtained

s not react with nitrous acid to so derivative is formed which is ction has been used for the de- rotein hydrolysates.[10]

inc-2-carboxylic acid) was first ischer in 1902. This amino acid roteins, but is found in relatively cent). Its isolation from protein nn found that, after removal of roline could be precipitated as a rner complex, termed ammonium



Ammonium Reineckate

cid Chemistry to Protein Analysis

iscussion, reference has been made to e characteristic of the side chains of ce the nitroprusside test (sulfhydryl (guanidino group), the Pauly reaction teis reaction (phenols), and the Mil- these reactions are also given by pro- amino acids on hydrolysis.[11,12] This in the unhydrolyzed protein, the side e not so substituted as to make them ion with these reagents. These color

J. Biol. Chem., 184, 607 (1950).
otein Chem., 3, 169 (1947).
Contrat. Chem. Revs., 41, 151 (1947).

reactions have long been used in qualitative tests for proteins in natural materials. It is of interest that protein-bound tryptophan does not respond to the Hopkins-Cole reaction.

Some of these reactions have also been used to good advantage in attempts to study proteins in intact cells. Of especial interest in such cytochemical studies is the use of ultraviolet absorption spectroscopy for the determination of protein concentration in cells and tissues. Since the ultraviolet absorption of protein solutions is of considerable practical value in protein studies, a brief discussion of the principles involved is desirable.

It will be recalled that the absorption of monochromatic light by a solution may be described by Beer's law, which states that the absorbancy, formerly termed optical density (d), of the solution is given by the expression $\log (I_0/I)$, where I_0 is the intensity of the incident light, and I is the intensity of the emergent light. The absorption coefficient (or extinction coefficient) E is defined as the optical density for a light path (l) of 1 cm; thus, $E = d/l$. If one wishes to express the light absorption in terms of the molar concentration (c) of the absorbing material in solution, the molar absorption coefficient (E_{mol}) is given by the equation $E_{mol} = E/c$. If the value of E (or E_{mol}) at various wave lengths of light is plotted as the ordinate against the wave length as the abscissa, a curve results which gives the absorption spectrum of the absorbing material in the solution. Most modern instruments designed for this purpose permit the accurate estimation of the optical density of a solution at narrowly spaced intervals from about 200 mμ (2000 A) to about 650 mμ (6500 A). [1 mμ = 10 A (angstrom units) = 10^{-7} cm.] Visible light is composed of light rays having wave lengths from about 400 mμ (violet) to about 650 mμ (red), and the region below 400 mμ is termed the ultraviolet region of the spectrum.

Most organic substances absorb light of wave lengths below 250 mμ; the absorption of light of longer wave lengths is usually associated with the presence, in the molecule, of unsaturated linkages. In general, an increase in the number of unsaturated linkages and their presence in conjugated systems contribute to light absorption at longer wave lengths. Of the widely distributed protein amino acids, only phenylalanine, tyrosine, and tryptophan exhibit extensive light absorption at wave lengths longer than 250 mμ; this may be attributed to their aromatic nature. The absorption spectra of these amino acids are given in Fig. 1, and it will be seen that phenylalanine exhibits maximal absorption at about 260 mμ, whereas tyrosine and tryptophan have their absorption maxima at about 275 mμ and 280 mμ, respectively. If a protein contains one or more of these amino acids, therefore, an aqueous solution of the protein

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will absorb light in the region 260 to 290 mμ, and this property may be used to measure its concentration. Since the relative proportions and absolute content of the three amino acids vary widely from one protein

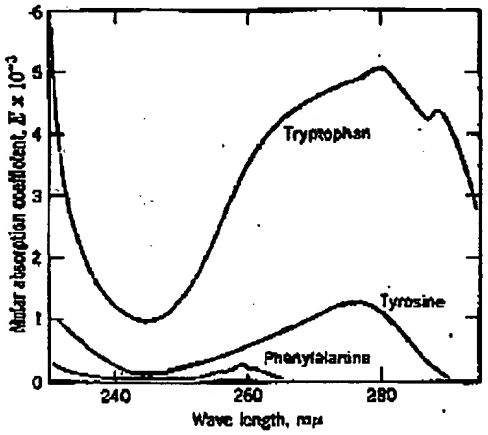

FIG. 1. Ultraviolet absorption spectra of aromatic amino acids derived from proteins (*p*H 8).

to another, each protein will, in general, exhibit a different value for the wave length of maximal absorption and the extinction coefficient. Thus, a one per cent solution of human serum albumin exhibits maximal absorption at 280 mμ, where the value of E is 5.32; on the other hand, a one per cent solution of beef insulin absorbs maximally at 277 mμ, and the value for E is 11.3. Clearly, the use of such values is justified only when one is dealing with solutions in which no other substances absorb light appreciably near 280 mμ.

Optical Activity of Amino Acids

Attention must now be given to the property of amino acids, when in solution that enables them to rotate the plane of polarized light. This property is termed optical activity. A brief outline of the basic concepts involved is given in what follows; a more complete discussion may be found in the treatise by Lowry.[18]

[18] T. M. Lowry, *Optical Rotatory Power*, Longmans, Green and Co., London, 1935.

Optical Activity of Amino

In 1669 it was found that
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image results. This was ex
who showed that when a ray
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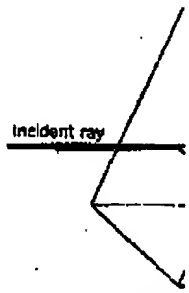

FIG. 2. Double refractio

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During the early part of the
Biot found that quartz crystals
the plane of the polarized ligh